AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph beginning at page 4, line 18 with the following amended paragraph:

Provided herein are methods for generating modified cytokines based on structural homology (3D scanning). These methods are based on the spatial and topological structure; they are not based on their underlying sequences of amino acid residues. The methods are used for identification of target sites for mutagenesis, particularly in families of target proteins. The targets are identified through comparison of patterns of protein backbone folding between and among structurally related proteins. The methods are ~~exemplifed~~ exemplified herein for cytokines. Families of the modified cytokines also are provided herein.

Please replace the paragraph beginning at page 6, line 18 with the following amended paragraph:

The modified cytokines have use as therapeutics. Each cytokine has improved biological and or therapeutic activity, compared to the know activity of the unmodified cytokine. Accordingly, uses of the cytokines for treatment of cytokine-mediated diseases and diseases for which immunotherapy is employed are provided. Methods of treatment using the modified cytokines for diseases also are provided. Each cytokine has a known therapeutic use, and such use is contemplated herein. ~~Cyokines~~ Cytokines provided herein have improved properties, such as increased bioavailability, improved stability, particularly *in vivo*, and/or greater efficacy.

Please replace the paragraph beginning at page 8, line 27 with the following amended paragraph:

Figure 6(G) provides graphs indicating the *in vitro* potency for antiviral activity, for IFNα-2b variants produced in bacteria. The vertical axis indicates the level of antiviral activity and the horizontal axis indicates concentration of the variants at which each level of activity is achieved. The activity for the variants (~~continous~~ continuous line with gray circles) was compared to that of the wild-type IFNα-2b (black triangles with dashed lines). The potency for each variant was calculated from the graphs as the concentration at the inflection point of the respective curves. Figure 6(T) shows the value of potency obtained for each variant tested compared to the wild type IFNα.

Please replace the paragraph beginning at page 9, line 15 with the following amended paragraph:

Figures 6(I) to 6(N) provide graphs indicating the pharmacokinetics in mice following subcutaneous injection of IFNα-2b variants produced in bacteria. The vertical axis indicates the level of antiviral activity in blood and the horizontal axis indicates the time after injection at which the level of antiviral activity is determined. The pharmacokinetics of the variants (in gray solid circles with gray continuous lines) was compared to that of the wild-type IFNα-2b (in black with dashed lines) and of a pegylated derivative (Pegasys, Roche) (36 μg/ml open triangles with continuous black lines; and 18 μg/ml open circles with continuous black lines); and vehicle (gray solid triangles with ~~continous~~ continuous gray lines. The Area Under the Curve (AUC) for each variant was calculated from the graphs and is shown in 6(U).

Please replace the paragraph beginning at page 16, line 1 with the following amended paragraph:

As used herein, "a directed evolution method" refers to methods that "adapt" either natural proteins, synthetic proteins or protein domains to work in new or existing natural or artificial chemical or biological environments and/or to elicit new functions and/or to increase or decrease a given activity, and/or to modulate a given feature. Exemplary directed evolution methods include pure random ~~mutageneis~~ mutagenesis methods; restricted random mutagenesis methods; and non-restricted rational mutagenesis methods, such as the rational directed evolution method described in co-pending U.S. application Serial No. 10/022,249; and the 2-dimensional rational scanning method provided herein.

Please replace the paragraph beginning at page 22, line 4 with the following amended paragraph:

Likewise, existing proteins known in the art that have previously been modified to have a desired increase or decrease in a particular biological activity compared to an unmodified reference protein can be selected and used herein for identification of structurally homologous loci on other structurally homologous target proteins. For example, a protein that has been modified by one or more single amino acid changes and possesses either an increase or decrease in a desired activity, such as resistance to proteolysis, can be utilized with the methods provided

herein to identify on structurally homologous target proteins, corresponding structurally homologous loci that can be replaced with ~~suitabale~~ suitable replacing amino acids and tested for either an increase or decrease in the desired biological ~~actiity.~~activity.

Please replace the paragraph beginning at page 30, line 22 with the following amended paragraph:

As used herein, families of non-related proteins or "sequence-non-related proteins" refers to proteins that have less than 50%, less than 40%, less than 0%, less [[thant]] than 20% amino acid identity or homology with each other.

Please replace the paragraph beginning at page 39, line 22 with the following amended paragraph:

Rational mutagenesis is a two-step process and is described in co-pending U.S. application Serial No. 10/022,249. Briefly, the first step requires amino acid scanning where all and each of the amino acids in the starting protein sequence are replaced by a third amino acid of reference (e.g., alanine). Only a single amino acid is replaced on each protein molecule at a time. A collection of protein molecules having a single amino acid replacement is generated such that molecules differ from each other by the amino acid position at which the replacement has taken place. Mutant DNA molecules are designed, generated by mutagenesis and cloned individually, such as in addressable arrays, such that they are physically separated from each other and such that each one is the single product of an independent mutagenesis reaction. Mutant protein molecules derived from the collection of mutant nucleic acid molecules also are physically separated from each other, such as by formatting in addressable arrays. Activity assessment on each protein molecule allows for the identification of those amino acid positions that result in a drop in activity when replaced, thus indicating the involvement of that particular amino acid position in the protein's biological activity and/or conformation that leads to fitness of the particular feature being evolved. Those amino acid positions are referred to as HITs. At the second step, a new collection of molecules is generated such that each molecule differs from each of the others by the amino acid present at the individual HIT positions identified in step 1. All 20 amino acids (19 remaining) are introduced at each of the HIT positions identified in step 1; while each individual molecule contains, in principle, one and only one amino acid

replacement. Mutant DNA molecules are designed, generated by mutagenesis and cloned individually, such as in [[in]] addressable arrays, such that they are physically separated from each other and such that each one is the single product of an independent mutagenesis reaction. Mutant protein molecules derived from the collection of mutant DNA molecules also are physically separated from each other, such as by formatting in addressable arrays. Activity assessment then is individually performed on each individual mutant molecule. The newly generated mutants that lead to a desired alteration (such as an improvement) in a protein activity are referred to as LEADs. This method permits an indirect search for activity alteration, such as improvement, based on one rational amino acid replacement and sequence change at a single amino acid position at a time, in search of a new, unpredicted amino acid sequence at some unpredicted regions along a protein to produce a protein that exhibits a desired activity or altered activity, such as better performance than the starting protein.

Please replace the paragraph beginning at page 41, line 30 with the following amended paragraph:

In particular embodiments, based on i) the particular protein properties to be evolved, ii) the protein's amino acid sequence, and iii) the known properties of the individual amino acids, a number of target positions along the protein sequence are selected, in silico, as "is-HIT target positions." This number of is-HIT target positions is as large as possible such that all reasonably possible target positions for the particular feature being evolved are included. In particular, embodiments where a restricted number of is-HIT target positions are selected for replacement, the amino acids selected to replace the is-HIT target positions on the particular protein being optimized can be either all of the remaining 19 amino acids or, more frequently, a more restricted group comprising selected amino acids that are contemplated to have the desired effect on protein activity. In another embodiment, so long as a restricted number of replacing amino acids are used, all of the amino acid positions along the protein backbone can be selected as is-HIT target positions for amino acid replacement. Mutagenesis then is performed by the replacement of single amino acid residues at specific is-HIT target positions on the protein backbone (e.g., "one-by-one," such as in addressable arrays), such that each individual mutant generated is the single product of each single mutagenesis reaction. Mutant DNA molecules are designed,

generated by mutagenesis and cloned individually, such as in addressable arrays, such that they are physically separated from each other and that each one is the single product of an independent mutagenesis reaction. Mutant protein molecules derived from the collection of mutant DNA molecules also are physically separated from each other, such as by formatting in [[in]] addressable arrays. Thus, a plurality of mutant protein molecules are produced. Each mutant protein contains a single amino acid replacement at only one of the is-HIT target positions. Activity assessment is then individually performed on each individual protein mutant molecule, following protein expression and measurement of the appropriate activity. An example of practice of this [[this]] method is shown in the Example in which mutant IFNα molecules and IFNβ ~~moleucles~~ molecules are produced.

Please replace the paragraph beginning at page 51, line 24 with the following amended paragraph:

The outcome of these two steps set forth above, which is performed *in silico* is that: (1) the amino acid positions that will be the target for mutagenesis are identified; these positions are referred to as is-HITs; (2) the replacing amino acids for the original, such as native, amino acids at the is-HITs are identified, to provide a collection of candidate LEAD mutant molecules that are expected to perform different from the native one. These are [[are]] assayed for a desired optimized (or improved or altered) biological activity.

Please replace the paragraph beginning at page 55, line 23 with the following amended paragraph:

Amino acids at the is-HITs then are replaced by residues that render the sequence less vulnerable (by a factor, for example, of 1%, 10%, 20%, 30%, 40%, 50%, . . . 100% depending upon the protein) or invulnerable (substantially no detectable digestion within a set time period) to protease digestion, while at the same time maintain a biological activity or activities of interest of the protein. The choice of the replacing amino acids is complicated by (1) the broad target specificity of certain proteases and (2) the need to preserve the physicochemical properties such as hydrophobicity, charge and polarity, of essential (e.g., catalytic, binding and/or other activities depending upon the protein) residues. For use in the methods herein, the "Percent Accepted Mutation" values (PAM values; see, Dayhoff et al., *Atlas of Protein Sequence and Structure*,

5(3):345-352, 1978), FIG2) can be used as ~~desribed~~ described herein. PAM values, originally developed to produce alignments between protein sequences, are available in the form of probability matrices, which reflect an evolutionary distance. Since, in a family of proteins or homologous (related) sequences, identical or similar amino acids (85% similarity) are shared, conservative substitutions for, or "allowed point mutations" of the corresponding amino acid residues can be determined throughout an aligned reference sequence. As noted, conservative substitutions of a residue in a reference sequence are those substitutions that are physically and functionally similar to the corresponding reference residues e.g., that have a similar size, shape, electric charge, chemical properties, including the ability to form bonds such as covalent and hydrogen bonds. For example, conservative substitutions can be those that exhibit the highest scores and fulfill the PAM matrix criteria in the form of "accepted point mutations."

Please replace the paragraph beginning at page 63, line 24 with the following amended paragraph:

Using this method, the following mutants selected as LEADs are provided herein and correspond to the group of proteins containing one or more single amino acid replacements in SEQ ID NO:1, corresponding to: F by V at position 27; R by H at position 33; E by Q at position 41; E by H at position 41; E by Q at position 58; E by H at position 58; E by Q at position 78; E by H at position 78; Y by H at position 89; E by Q at position 107; E by H at position 107; P by A at position 109; L by V at position 110; M by V at position 111; E by Q at position 113; E by H at position 113; L by V at position 117; L by I at position 117; K by Q at position 121; K by T at position 121; R by H at position 125; R by Q at position 125; K by Q at position 133; K by T at position 133; and E by Q at position 159; E by H at position 159. Among these are mutations that can have multiple effects. For ~~exmple,~~example, among mutations described herein, are mutations that result in an increase of the IFNα-2b activity as assessed by detecting the requisite biological activity.

Please replace the paragraph beginning at page 81, line 8 with the following amended paragraph:

In an exemplary embodiment herein, IFNα-2b mutants with increased resistance to proteolysis are generated by the 2-dimensional rational scanning method; IFNβ mutants also

were generated. The corresponding residues on members of cytokine families that possess structural homology to IFNα-2b were identified and the ~~identifeid~~ identified residues on the other cytokines were similarly modified to produce cytokines with increased resistance to proteolysis. Hence also provided herein are cytokine mutants that display increased resistance to proteolysis and/or glomerular filtration containing one or more amino acid replacements.

Please replace the paragraph beginning at page 99, line 5 with the following amended paragraph:

By 3D-scanning (see, SEQ ID Nos.: 234-289, 989-1015): D by Q at position 39, D by H at position 39, D by G at position 39, E by Q at position 42, E by H at position 42, K by Q at position 45, K by T at position 45, K by S at position 45, K by H at position 45, L by V at position 47, L by I at position 47, L by T at position 47, L by Q at position 47, L by H at position 47, L by A at position 47, K by Q at position 52, K by T at position 52, K by S at position 52, K by H at position 52, F by I at position 67, F by V at position 67, R by H at position 71, R by Q at position 71, D by H at position 73, D by G at position 73, D by Q at position 73, E by Q at position 81, E by H at position 81, E by Q at position 107, E by H at position 107, K by Q at position 108, K by T at position 108, K by S at position 108, K by H at position 108, E by Q at position 109, E by H at position 109, D by Q at position 110, D by H at position 110, D by G at position 110, F by I at position 111, F by V at position 111, R by H at position 113, R by Q at position 113, L by V at position 116, L by I at position 116, L by T at position 116, L by Q at position 116, L by H at position 116, L by A at position 116, L by V at position 120, L by I at position 120, L by T at position 120, L by Q at position 120, L by H at position 120, L by A at position 120, K by Q at position 123, K by T at position 123, K by S at position 123, K by H at position 123, R by H at position 124,[[,]] R by Q at position 124, R by H at position 128, R by Q at position 128, L by V at position 130, L by I at position 130, L by T at position 130, L by Q at position 130, L by H at position 130, L by A at position 130, K by Q at position 134, K by T at position 134, K by S at position 134, K by H at position 134, K by Q at position 136, K by T at position 136, K by S at position 136,, K by H at position 136, E by Q at position 137, E by H at position 137, Y by H at position 163, Y by I at position [[163I,]]163, R by H at position 165, R by Q at position 165.

Please replace the paragraph beginning at page 117, line 15 with the following amended paragraph:

In particular, the optimized cytokines, such as the IFNα-2b and IFNβ proteins, are intended for use in therapeutic methods in which cytokines have been used for treatment. Such methods include, but are not limited to, methods of treatment of infectious diseases, allergies, microbial diseases, pregnancy related diseases, bacterial diseases, heart diseases, viral diseases, histological diseases, genetic diseases, blood related diseases, fungal ~~diseses,~~diseases, adrenal diseases, cancers, liver diseases, autoimmune diseases, growth disorders, diabetes, neurodegenerative diseases, including ~~mulitiple~~ multiple sclerosis, Parkinson's disease and Alzheimer's disease.

Please replace the paragraph beginning at page 118, line 19 with the following amended paragraph:

The mutant cytokines ~~inleuding~~ including the mutant interferons (IFNα's and [[IFNβ')]] IFNβ's) proteins provided herein, also can be delivered to the cells in gene transfer vectors. The transfer vectors also can encode [[encode]] additional other therapeutic agent(s) for treatment of the disease or disorder, such cancer or HIV infection, for which the cytokine is administered.

Please replace the paragraph beginning at page 122, line 8 with the following amended paragraph:

The compositions, if desired, can be presented in a package, in kit [[a or]] or a dispenser device, that can contain one or more unit dosage forms containing the active ingredient. The package, for example, contains metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration. The compositions containing the active agents can be packaged as articles of manufacture containing packaging material, an agent provided herein, and a label that indicates the disorder for which the agent is provided.

Please replace the Table beginning at page 122, line 26 with the following amended Table:

Cytokine	Exemplary Uses, Diseases and Treatment

IL-10	anti-inflammatory treatment of chronic liver injury and disease; myeloma
Interferon-gamma	Interstitial/idiopathic pulmonary fibrosis; adjunctive immunotherapy for ~~immunosupressed~~ immunosuppressed patients
Granulocyte colony stimulating factor	Crohn's disease; cardiac disease; acquired and congenital neutropenias; asthma
Leukemia inhibitory factor	myocardial infarction; multiple sclerosis; prevention of axonal atrophy;olfactory epithelium replacement stimulation
Human growth hormone	growth hormone deficiency; acromegaly
Ciliary neurotrophic factor	retinal degeneration treatments; ~~neurodegnerative~~ neurodegenerative diseases such as ~~Huntingtons;~~ Huntington's; auditory degenerative diseases
Leptin	obesity; pancreatitis; ~~endometreosis~~ endometriosis
Oncostatin M	chronic ~~infammatory~~ inflammatory diseases; rheumatoid arthritis; multiple sclerosis; tissue damage ~~supression~~ suppression
Interleukin-6	Protection from liver injury; Crohn's disease; hematopoietic associated diseases
Interleukin-12	~~coksakievirus~~ coxsackievirus treatment;neuroblastoma; melanoma, renal cell carcinoma; mucosal immunity induction
Erythropoietin	hypoxia; myocardial ischemia; anemia with renal failure and cancer treatments
Granulocyte-macrophage colony stimulating factor	stimulate antigen presenting cells; anti-tumor activity for leukemia, melanoma, and breast, liver and renal cell carcinomas; adjunctive immunotherapy for

	~~immunosupressed~~ immunosuppressed patients; ~~automimmune~~ autoimmune disease
Interleukin-2	immune reactivation after chemotherapy; melanoma; colon carcinoma
Interleukin-3	leukemia cell targeting; motor neuropathy; amyotrophic lateral sclerosis; asthma
Interleukin-4	allergic asthma; lupus
Interleukin-5	treatment for parasites;asthma; allergic diseases accompanied by eosinophilia
Interleukin-13	intracellular infections; B-cell cancers; asthma
Flt3 ligand	prostate cancer; myeloid leukemia; engraftment of allogenic ~~hematopietic~~ hematopoietic stem cells
Stem cell factor	hepatic injury; asthma; hematopoietic engraftment

Please replace the paragraph beginning at page 124, line 30 with the following amended paragraph:

The IFNα-2b cDNA was first cloned into [[an]] a mammalian expression vector, prior to the generation of the selected mutations. A collection of mutants was then generated such that each individual mutant was created and processed individually, physically separated [[form]] from each other and in addressable arrays. The mammalian expression vector pSSV9 CMV 0.3 pA was engineered as follows:

Please replace the paragraph beginning at page 130, line 5 with the following amended paragraph:

IFNα-2b mutants were produced in 293 human embryo kidney (HEK) cells (obtained from ATCC), using ~~Dubelcco's~~ Dulbecco's modified Eagle's medium supplemented with glucose (4.5 g/L; Gibco-BRL) and fetal bovine serum (10%, Hyclone). Cells were transiently transfected with the plasmids encoding the IFNα-2b mutants as follows: 0.6×10^5 cells were seeded into 6 well-plates and grown for 36 h before transfection. Confluent cells at about 70% were supplemented with 2.5 μg of plasmid (IFNα-2b mutants) and 10 mM poly-ethylene-imine (25 KDa PEI, Sigma-Aldrich). After gently shaking, cells were incubated for 16 h. Then, the

culture medium was changed with 1 ml of fresh medium supplemented with 1% of serum. IFNα-2b was measured on culture supernatants obtained 40 h after transfection and stored in aliquots at -80°C until use.

Please replace the paragraph beginning at page 132, line 4 with the following amended paragraph:

D. Screening and *in vitro* ~~charaterization~~ characterization of IFN α-2b mutants

Two activities were measured directly on IFN samples: antiviral and antiproliferation activities. Dose (concentration) - response (activity) experiments for antiviral or antiproliferation activity permitted calculation of the "potency" for antiviral and antiproliferation activities, respectively. Antiviral and antiproliferation activities also were measured after incubation with proteolytic samples, such as specific proteases, mixtures of selected proteases, human serum or human blood. Assessment of activity following incubation with proteolytic samples allowed to determine the residual (antiviral or antiproliferation) activity and the respective kinetics of half-life upon exposure to proteases.

Please replace the paragraph beginning at page 134, line 15 with the following amended paragraph:

Anti-proliferative activity of interferonα-2b was determined by the capacity of the cytokine to inhibit proliferation of Daudi cells. Daudi cells (1 x 10^4 cells) were seeded in flat-bottomed 96-well plates containing 50 µl/well of RPMI 1640 medium supplemented with 10% SVF, 1X ~~glutamin~~ glutamine and 1ml of gentamicin. No cell was added to the last row ("H" row) of the flat-bottomed 96-well plates in order to evaluate background absorbance of culture medium.

Please replace the paragraph beginning at page 136, line 19 with the following amended paragraph:

IFNα-2b mutants selected on the basis of their overall performance *in vitro*, were tested for pharmacokinetics in mice in order to have an indication of their half-life in blood *in vivo*. Mice were treated by subcutaneous (SC) injection with aliquots of each of a number of selected lead mutants. Blood was collected at increasing time points between 0.5 and 48 hours after injection. ~~Inmediatedly~~Immediately after collection, 20 ml of anti-protease solution were added

to each blood sample. Serum was obtained for further analysis. Residual IFN-α activity in blood was determined using the tests described in the precedent sections for *in vitro* characterization. Wild-type IFN α (that had been produced in bacteria under comparable conditions as the lead mutants) as well as a pegylated derivative of IFN α, Pegasys (Roche), also were tested for pharmacokinetics in the same experiments.

Please repiace the paragraph beginning at page 150, line 26 with the following amended paragraph:

IFN β was produced in CHO Chinese Hamster Ovarian cells (obtained from ATCC), using ~~Dubelcco's~~ Dulbecco's modified Eagle's medium supplemented with glucose (4.5 g/L; Gibco-BRL) and fetal bovine serum (5 %, Hyclone). Cells were transiently transfected as follows: 0.6 x 10^5 cells were seeded into 6 well plates and grown for 24 h before transfection. Confluent cells at about 70%, were supplemented with 1.0 µg of plasmid (from the library of IFN β mutants) by lipofectamine plus reagent (Invitrogen). After gently shaking, cells were incubated for 24 h with 1 ml of culture medium supplemented with 1 % of serum. IFN β was obtained from culture supernatants 24 h after transfection and stored in aliquots at -80°C until use.

Please replace the paragraph beginning at page 151, line 5 with the following amended paragraph:

Preparations of IFN β produced from transfected cells were screened following sequential biological assays as follows. Normalization of IFN β concentration from culture supernatants was performed by ELISA. IFN β concentrations from wild type, and ~~mutants~~ mutant samples were estimated by using an international reference standard provided by the NIBSC, UK.

Please replace the paragraph beginning at page 152, line 6 with the following amended paragraph:

After 24 hours of growth, a 1/1000 EMC virus dilution solution was placed in each well, except for the cell control row. Plates were returned to the CO_2 incubator for 48 hours. Then, the medium was aspirated and the cells were stained for 1 hour with 100 µl of Blue staining ~~solutio~~solution to determine the proportion of intact cells. Plates were washed in a distilled water bath. The cell bound dye was extracted using 100 µl of ethylene-glycol mono-ethyl-ether

(Sigma). The absorbance of the dye was measured using an Elisa plate reader (Spectramax). The antiviral activity of IFN β samples (expressed as number of IU/mg of proteins) was determined as the concentration needed for 50% protection of the cells against EMC virus-induced cytopathic effects. For proteolysis experiments, each point of the kinetic was assessed at 800 and 400 pg/ml in triplicate.

Please replace the paragraph beginning at page 154, line 26 with the following amended paragraph:

By 3D-scanning (see, SEQ ID Nos. 234-289, 989-1015): D by Q at position 39, D by H at position 39, D by G at position 39, E by Q at position 42, E by H at position 42, K by Q at position 45, K by T at position 45, K by S at position 45, K by H at position 45, L by V at position 47, L by I at position 47, L by T at position 47, L by Q at position 47, L by H at position 47, L by A at position 47, K by Q at position 52, K by T at position 52, K by S at position 52, K by H at position 52, F by I at position 67, F by V at position 67, R by H at position 71, R by Q at position 71, D by H at position 73, D by G at position 73, D by Q at position 73, E by Q at position 81, E by H at position 81, E by Q at position 107, E by H at position 107, K by Q at position 108, K by T at position 108, K by S at position 108, K by H at position 108, E by Q at position 109, E by H at position 109, D by Q at position 110, D by H at position 110, D by G at position 110, F by I at position 111, F by V at position 111, R by H at position 113, R by Q at position 113, L by V at position 116, L by I at position 116, L by T at position 116, L by Q at position 116, L by H at position 116, L by A at position 116, L by V at position 120, L by I at position 120, L by T at position 120, L by Q at position 120, L by H at position 120, L by A at position 120, K by Q at position 123, K by T at position 123, K by S at position 123, K by H at position 123, R by H at position 124,, R by Q at position 124, R by H at position 128, R by Q at position 128, L by V at position 130, L by I at position 130, L by T at position 130, L by Q at position 130, L by H at position 130, L by A at position 130, K by Q at position 134, K by T at position 134, K by S at position 134, K by H at position 134, K by Q at position 136, K by T at position 136, K by S at position 136,, K by H at position 136, E by Q at position 137, E by H at position 137, Y by H at position 163, Y by I at position [[163I,]] 163, R by H at position 165, R by Q at position 165.